

Mechanistic examination of causes for narrow distribution in an endangered shrub: A
comparison of its responses to drought stress with a widespread congeneric species

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ABSTRACT

Although deep rooting is usually considered a drought-tolerant trait, we found *Syringa pinnatifolia*, a deep-rooting and hydrotropic shrub, has a limited distribution in arid areas. In order to elucidate the mechanisms for its narrow distribution, we conducted two experiments to examine the physiological and morphological responses to water availability and heterogeneity in *S. pinnatifolia* and a widespread congeneric species, *S. oblata*. We measured gas exchange, water use efficiency and plasticity index in plants of these two species grown at different levels of soil water regimes and in containers with patched water distribution. Our results showed that high photosynthetic capacity in the narrowly distributed *S. pinnatifolia* was an important factor enabling its survival in the harsh sub-alpine environment. High photosynthetic capacity in *S. pinnatifolia*, however, was obtained at the expense of high transpiratory water loss, resulting in lower integrative water use efficiency. Biomass allocation to roots in *S. pinnatifolia* increased by 73% when soil water increased from 75% to 95% field capacity, suggesting that *S. pinnatifolia* could be less competitive for above-ground resources under favorable water regimes. The horizontal and vertical root hydrotropism of *S. pinnatifolia* in soil with patched water patterns were likely related to compensation for leaf water loss at low soil water level, indicating a limited capacity for homeostasis within the plant for water conservation and lower level of inherent drought-tolerance. In summary, greater degree of morphological plasticity but lower degree of physiological adjustment may be the main causes for the hydrotropism and narrow distribution of *S. pinnatifolia* in the sub-alpine habitats.

Key words: endangered species; hydrotropism; lilac; photosynthesis; soil water; *Syringa*.

Key Message

The endangered status and narrow distribution in *Syringa pinnatifolia*, a sub-alpine shrub species, was primarily caused by its poor drought adaptation related to its lower degree of physiological adjustment despite its greater degree of morphological plasticity.

INTRODUCTION

Syringa species are functionally and morphologically diverse shrubs and have long been valued and used by humans worldwide. The wild germplasms of *Syringa* mainly occur from low-lying areas to sub-alpine mountains of northern and south-western China (Chang and Qiu, 1992). One of the most narrowly distributed and endangered species of *Syringa* is *S. pinnatifolia* (Fu and Zhang, 1992), the only *Syringa* species with pinnated leaves and distributed only in mountains between an elevation of 1,700 and 3,100 m. It is particularly susceptible to drought stress because of its extravagant requirement for water supply for normal growth as found in our *ex situ* conservational studies despite its deep roots (unpublished data). The congeneric *S. oblata* is more broadly distributed in arid area across southwestern, northwestern and northeastern China (Chang and Qiu, 1992). Although these two species are found in the same regions, their habitats are generally non-overlapping with *S. oblata* occurring at lower elevation and *S. pinnatifolia* at higher elevation. Unlike *S. pinnatifolia* which can root to a depth of 0.90 m, *S. oblata* is shallow rooting with its roots confined to a soil depth of 0.25 m, but found to be tolerant of drought and low temperature when grown in gardens and in its native habitats (personal observations). Because of the physiological and morphological contrasts in these two *Syringa* species, it would be interesting to examine the mechanisms that *S. pinnatifolia* uses to adapt to drought stress in its native habitats and compare its responses to drought with the more widespread *S. oblata*.

Drought and moisture heterogeneity occur commonly in soils in arid and semiarid regions due to seasonality, topography and uneven precipitation (Loik et al., 2004). Most

native plants have adapted to the limited water supply and shifting soil moisture by evolving physiological and morphological strategies in their habitats. These strategies include reduction in water loss through efficient stomatal regulation, enhancement of water use efficiency, decrease in xylem hydraulic conductivity, formation of hydrotropism and increase in water acquisition through root systems (Nicotra et al., 2002; North and Nobel, 2000; Schuster et al., 1992; Vilagrosa et al., 2003). Narrowly distributed plant species are typically characterized by stronger selection for spatiotemporal heterogeneity (Valladares et al., 2007). Accordingly, these species have narrower ecological niches and higher cost in attaining given phenotypes in response to environmental stresses, resulting in inferior competitiveness and low fitness relative to more widespread species (Alvarez-Yepiz et al., 2011; Matthies et al., 2004; Ohlemuller et al., 2008; Partel et al., 2004). Regardless of their distribution ranges, both narrowly distributed and widespread species adapt to drought through physiological and morphological coordination (Drouet and Pages, 2003; Givnish, 2002). The most fundamental differences between these two groups lie only in the strategies and cost of attaining these strategies.

In arid sub-alpine regions, optimization between water conservation and photosynthetic assimilation is more imperative than competitive photosynthetic capacity for sustainable surviving and spreading (Gutschick and BassiriRad, 2003). Active photosynthesis is essential to provide energy for vital functions such as cold acclimation (Huner et al., 1998), but would not be beneficial if high photosynthetic capacity comes at the expense of greater water loss, particularly in arid areas or during arid seasons. Optimal water use efficiency is thus crucial

through optimization of carbon gain and water loss, i.e., a trade-off between photosynthetic carbon assimilation and transpirational water loss (Peleg et al., 2005). Optimal water use efficiency is achieved through the coordination between foliar transpiration control and root uptake of water is an adaptive response to drought and moisture heterogeneity in the soil, similar to coordination between leaf turgor maintenance capacity and stem xylem cavitation resistance under drought conditions (Fu et al., 2012). Many drought-tolerant species with shallow roots survive drought through homeostasis within leaves involving stomatal closure in response to low leaf water potential (Davis, 1989; Davis and Mooney, 1986; Rambal, 1993). In contrast, deep rooting, a form of hydrotropism to alleviate drought stress by accessing water deep in the soil, has been considered morphological drought avoidance instead of drought tolerance (Cassab et al., 2013; Nepstad et al., 1994).

Plasticity costs in plants seem to have more impact on survival in stressed environments than under favorable conditions with regular resource supply (Steinger et al., 2003). Physiological plasticity has been shown to be more cost-effective than morphological adjustments to a certain extent, especially when plants need to utilize transient resources (de Witt et al., 1998; Godoy et al., 2012; Grams and Anderson, 2007). Higher cost of acquisition and processing of resource information could lead to a lower fitness in phenotypes with a low degree of adaptation, particularly when misperceptions of resource variation and time-lag construction happen (de Witt et al., 1998; Valladares et al., 2007). It is unclear, however, if and how plastic strategies differ between narrowly distributed and widespread species of close taxonomy.

In the present study, we examined the differences between the physiological and morphological responses to elevated water regimes and heterogeneous soil water distribution in the narrowly distributed *S. pinnatifolia* and widely distributed *S. oblata*. We were keenly interested in whether *S. pinnatifolia* has directional hydrotropism and if it does, how directional hydrotropism would affect its drought survival and drought adaptation in relation to its endangered status. We formulated four hypotheses in this comparative study to examine the underlying mechanisms as to why one *Syringa* species is narrowly distributed and the other is more widespread. Firstly, photosynthetic capacity, long-term integrative water use efficiency and root hydraulic conductivity would be lower in *S. pinnatifolia* than in *S. oblata* across elevated water regimes. Secondly, *S. pinnatifolia* would allocate less biomass to belowground and thus make it less competitive for water resources in its native habitats. Thirdly, the more widely distributed *S. oblata* would be more flexible in biomass allocation in heterogeneous water patches to make it more adaptive to the arid environment. Finally, we hypothesized that it was the differences in physiological and morphological plasticity and in hydrotropism between the two species make one species narrowly distributed and the other widespread.

MATERIALS AND METHODS

Seeds of *S. pinnatifolia* and *S. oblata*, which are deciduous bushes or small trees, were collected in Hohhot Botanical Garden, Inner Mongolia and Beijing Botanical Garden, respectively, in 2005. Seeds were sowed and germinated in terra cotta pots (20 cm in diameter

and height) filled with a 2:1:1 mixed soil of peat, sand and vermiculite on the campus of the Institute of Botany, Chinese Academy of Science, Beijing, China (39°9'N, 116°4'E) in 2006. In March 2007, seedlings were removed from the pots and weighed for initial biomass before being transplanted back into the pots in preparation for the experiments.

Experimental design

Experiment 1 - Response to elevated water supply. The first experiment was conducted from May 1 to September 30, 2007, to examine the physiological and growth responses of the two *Syringa* species to different levels of soil water. Uniform one-year-old seedlings, which had been grown since March 2007 in terra cotta pots (20 cm in diameter and height) filled with a 1:1 mixture of peat and top soil, were used in this experiment. The water treatments were 35, 55, 75 and 95% of the maximum field capacity (FC_{max}) with five replications. All seedlings were shielded from rain with plastic cloth to avoid disturbance to the water treatments during the experiment. The amount of water supply was determined by the difference between the target weight at the required water content and the actual weight after water loss through transpiration. Lost water was supplemented in each pot every three to four hours from 0600 h to 1800 h to keep water content at the targeted level. Plant leaves in all treatments remained green during the experiment, although those grown at low water levels showed some signs of drought stress. Pots were covered on the soil surface with cardboard to reduce water evaporation. No water leakage was observed during the experiments because of the moderate water quantity added at a high frequency. Mean air temperature during the experiments was 30-33°C during daytime and 20°C at nighttime. Relative humidity was maintained at 50-70%

during daytime and the maximum photosynthetically active radiation (PAR) was 1500-1900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on clear days during the experiments. This experiment was repeated from June 1 to October 20, 2009, but only for water levels of 75% and 95% FC_{max} to conform the unexpected results of biomass allocation in *S. pinnatifolia* found in the initial experiment at these two water levels.

Experiment 2 - Response to patched water distribution. This experiment was conducted at the same time as the first part of Experiment 1 in the summer of 2007 to examine how the two *Syringa* species differed in response to water heterogeneity in the soil. Three cardboard boxes ($40 \times 25 \times 30$ cm in dimension) were filled with the same growth medium as used in Experiment 1 (Fig. 1). Each box was divided vertically into three equal parts. The middle part was filled with growth medium that was well-watered (97-100% of FC_{max}) and the two end parts were filled with soil of approximately 40% of FC_{max} to simulate water-stressed conditions. One individual of *S. pinnatifolia* was grown in one water-stressed end and another individual of *S. oblata* was grown in the opposite water-stressed end. Before each watering event, two thin fibreboards were inserted into the medium along the boundaries between the well-watered middle and the water-stressed end parts to prevent water movement between the sections. The boards were removed after watering had been completed. No evidence of significant root damage by insertion and removal of fibreboard was observed at harvesting.

Physiological and morphological measurements

Gas exchange. Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) were measured during 0800 h-1200 h in the morning monthly using a portable gas

exchange system (LI-6400, Licor Inc., Lincoln, NE, USA) throughout the experiment.

Temperature, relative humidity and radiation intensity in the leaf cuvette were set at ambient conditions.

Integrative water use efficiency. Seasonal integrative water use efficiency (WUE_I) was calculated as the ratio of biomass increment to the total amount of water supplemented for each seedling. Initial and final biomass was measured at the beginning and end of the experiments to obtain biomass increment in plants grown under different water treatments. Water consumption by plants was calculated by adding up the total amount of water supplemented during the experiment.

Root hydraulic conductivity. Root hydraulic conductivity (L_{pr}) was determined at the end of Experiment 1 using the pressure chamber method as described in Huxman et al. (1999). The whole root system with soil was put into the pressure chamber (PMS Instruments, Albany, Oregon, USA) after the above-ground part was pruned. Pressure was reduced from 1.4 to 0.2 MPa at an interval of 0.2 MPa. Three minutes were required to get a stable sap outflow at each pressure level. Pre-weighted filter paper was used to collect effused sap on the root section one minute after stabilization of sap flow at a given pressure. The quantity of sap obtained from each pressure level was calculated as the weight difference in the filter paper before and after sap collection. After sap collection, roots were washed clean of soil, air dried and scanned using a Epson U700 Scanner (Epson, Japan) for estimating root surface area using image analysis software WinRHIZO (Regent, Canada). Root flow density (J_v) at a given pressure for each water regime was calculated as the ratio of sap weight to root surface

area. Hydraulic conductivity was subsequently obtained from the slope of the linear regression generated from curve of J_v against applied pressure.

Morphological measurement and plasticity index calculation. Biomass was determined at the beginning and end of the experiments. Roots, stems and leaves were separately harvested and dried in an oven at 108°C for half an hour to deactivate the enzymes. Plant tissues were further dried at 75°C until constant weight. In Experiment 2, soil columns were dissected in all three card boxes to examine root distribution patterns after five months of growth in habitats with heterogeneous water distribution. At the end of Experiment 2, water was slowly and evenly poured into each card box before the box was moved into an icebox to be frozen at -16°C for 12 hours. Each frozen box of soil was vertically sawed into three columns, i.e., one well-watered section and two water-stressed sections to examine root distribution. Soil columns were further dissected horizontally for each frozen column into layers of a, b, and c as shown in Fig. 1. As a result, nine blocks were obtained from each frozen box of soil. The soil blocks were then numbered and immersed in water at room temperature. After complete thawing, roots were collected from each block and later dried to determine the spatial distribution of root biomass. Plasticity indices for physiological and morphological parameters were calculated as the average of the difference between minimum and maximum measurement values across the four water treatments divided by the maximum value as described in Valladares et al. (2000).

Statistical analysis

Physiological and growth variables were all analyzed using one-way analysis of variance

(ANOVA) using SPSS (Ver. 11.5, SPSS, Chicago, USA) focusing on the mechanistic responses to four levels of water treatment in each species and how they differed in adaptation to drought stress. Differences were considered significant if $P < 0.05$.

RESULTS

Gas exchange, water transpiration and integrative water use efficiency

The endangered species, *S. pinnatifolia*, differed greatly from the widespread species, *S. oblata*, in photosynthetic response to increase in soil water availability (Table 1). For example, photosynthetic rate (P_n) of *S. pinnatifolia* increased steadily from 6.0 to 14.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ when water level increased from 35% to 95% of FC_{max} . By contrast, P_n of *S. oblata* increased significantly when water level increased from 35% to 55% FC_{max} , but dropped noticeably at 75% FC_{max} (Table 1). *S. pinnatifolia* had higher P_n than *S. oblata* at all water levels except at 55% FC_{max} (Table 1). Stomatal conductance (g_s) in leaves of *S. pinnatifolia* increased as soil water increased before leveling off at 75% FC_{max} (Table 1). Water transpiration did not differ between *S. pinnatifolia* and *S. oblata* except at 95% FC_{max} , when *S. pinnatifolia* had lower water transpiration than *S. oblata* (Table 1). Integrated water use efficiency (WUE_i), however, was much lower in *S. pinnatifolia* than in *S. oblata* (Table 1). There was a noticeable difference between the two species in the range of water supply required for greatest biomass production. For the endangered *S. pinnatifolia*, maximal biomass increment occurred at 75% of FC_{max} only, while for the widespread *S. oblata* maximal biomass growth was achieved at a much wider range of water levels (55% to 95%)

(Table1).

Root hydraulic conductivity

Root hydraulic conductivity (L_{pr}) in both *Syringa* species increased similarly with increasing supply of water (Fig. 2). The two species, however, differed in the magnitude of variation in L_{pr} across the range of water levels. L_{pr} varied to a lesser degree in *S. pinnatifolia* than in *S. oblata*. There was no significant difference in L_{pr} between the two species in water regimes of 35-75% FC_{max} , while *S. pinnatifolia* had much lower L_{pr} than *S. oblata* at 95% FC_{max} .

Biomass and biomass allocation

Stem biomass in the common *S. oblata* increased steadily as soil water increased from 35% to 95% FC_{max} (Fig. 3a). Stem biomass in the endangered *S. pinnatifolia* also increased from 35% to 75% FC_{max} , but declined when soil water was increased to 95% FC_{max} . The endangered *S. pinnatifolia* allocated a greater proportion of biomass to shoots than the widespread *S. oblata* at all water levels except at 95% FC_{max} (Fig. 3b). Allocation to roots in *S. pinnatifolia* was thus correspondingly lower than in *S. oblata* at water levels of 35%, 55%, and 75% FC_{max} (Fig. 4). Root fraction in *S. oblata* decreased gradually as water supply increased and was the lowest at 95% FC_{max} (Fig. 4). For *S. pinnatifolia*, however, root fraction decreased from 35% to 75% FC_{max} of soil water level, but increased from 75% to 95% FC_{max} (Fig. 4).

Pattern of root distribution

Roots of *S. oblata* were distributed symmetrically in the soil with 18.3% of the total root mass in the near-water area and 20.6% of the root mass in the soil far away from the water source. In contrast, *S. pinnatifolia* had strong tropism to water in terms of root distribution (Fig. 5).

While 47.6% of the root mass was distributed in near-water soil, only 22.7% of the root mass was found in the soil section far away from the water source. Vertical distribution of roots in these two species also differed greatly. *S. oblata* had a greater proportion of roots in the shallower soil layer, whereas *S. pinnatifolia* had a greater portion of roots in the deeper layer (Fig. 6).

Physiological plasticity

Physiological plasticity was generally lower in *S. pinnatifolia* than in *S. oblata*, particularly for transpiration, stomatal conductance and hydraulic conductivity (Table 2). In contrast, morphological plasticity was similar for the two species under elevated water regimes. Plasticity index was the highest for stem biomass and the lowest for root mass of all the morphological traits for both species in homogeneous water supply (Table 2). In heterogeneous water pattern, *S. pinnatifolia* and *S. oblata* did not differ much in shoot and root mass fractions (Fig. 7). In homogenous water pattern, *S. pinnatifolia* had a greater stem mass fraction and lower root mass fraction than in *S. oblata* (Fig. 7).

DISCUSSION

Carbon assimilation at the expense of greater water loss in S. pinnatifolia

The narrowly distributed *S. pinnatifolia* had comparable or even higher net photosynthetic rate than the widely spread *S. oblata* under most homogeneous water regimes. Despite its higher photosynthetic rate, individuals of *S. pinnatifolia* had lower biomass accumulation than those of *S. oblata* in our study, implying greater energy consumption in its surviving as found

in C₄ grass species grown under water-deficit conditions (Maroco et al., 2000). Furthermore, *S. pinnatifolia* had a much lower integrative water use efficiency (WUE_i), a result of lower biomass increment but similar amount of water transpiration, than the widespread *S. oblata*. These results suggest that photosynthetic rate may not be the primary reason causing *S. pinnatifolia* to be narrowly distributed. Most likely there are other reasons that combine to make it narrowly distributed and endangered. First, the adaptation of *S. pinnatifolia* to a harsh environment may have led to a trade-off that made it less competitive where *S. oblata* is distributed. Second, it might be the great amount of water needed for carbon assimilation and the greater energy consumption required for its survival under drought-stressed mountainous conditions. Our conclusion is supported by earlier finding showing that plants had greater energy cost for growth and survival under drought conditions (Maroco et al., 2000).

Our previous studies have demonstrated that stomata of *S. pinnatifolia* leaves had larger guard cells and stomatal aperture compared to *S. oblata* with the average stomatal size in leaves of *S. pinnatifolia* and *S. oblata* being 237 and 196 μm^2 , respectively (Gao et al., 2008). It has been known that instant transpiration adjustment in response to variation of external water conditions is largely determined by the size of stomata (Hetherington and Woodward, 2003). Smaller stomata can open and close more rapidly than larger ones (Aasamaa et al., 2001). Our results of higher water transpiration loss found in the narrowly-spread species relative to the widespread species imply that *S. pinnatifolia* may not have the capacity to adequately regulate stomatal opening because of the larger-size stomata. Earlier research has suggested that a moderate decrease in root conductivity entails a control on water

transportation in plants, particularly in those grown under fluctuating water availability (Jones, 1992). Our study, however, found that loss of root transport capability was not induced in the narrowly distributed *S. pinnatifolia*, although strong transpiration occurred in soils with moderate soil moisture (75% FC_{max}) to drought (35% FC_{max}) (Fig. 2). These results suggest that the inherent mechanism of transpiration regulation in leaves and roots of *S. pinnatifolia* might not have been set up optimally for controlling transpiratory water loss. The survival of *S. pinnatifolia* individuals will thus have to depend more on the water uptake via roots from external environment than homeostasis in foliar water conservation through stomatal regulation.

Non-optimal allocation of photosynthate in S. pinnatifolia

Seedlings of *S. pinnatifolia* had lower allocation of biomass to roots (23-51%) than those of the widespread *S. oblata* (36-68%) across all water regimes (Fig. 4). *S. pinnatifolia* instead allocated a greater portion of assimilate to growing stems for acquiring above-ground resources, even in soils that were relatively water-limiting, i.e., 35% FC_{max}. The lower allocation of biomass to roots will therefore hinder the compensation for its relatively higher water loss through leaves because of larger stomatal area.

It is interesting to note that *S. pinnatifolia* plants significantly increased biomass allocation to roots in the well-watered soil (95% FC_{max}) as first found in our 2007 experiment and later confirmed in our 2009 study. This allocation pattern is not consistent with the tendency of plants to allocate a greater proportion of photosynthate to above-ground components, particularly leaves, for maximizing light interception capability, when water is

non-limiting (Fisher and Turner, 1978). Root-dominated allocation of biomass in *S. pinnatifolia* during periods of abundant water supply demonstrate that this species has a non-optimal biomass allocation pattern. More biomass allocated to roots in plants grown under well-water conditions comes at the expense of construction of above-ground structure and will likely impair to acquire and utilize above-ground resource. These traits combine to reduce the ability of *S. pinnatifolia* to compete for resources and limit the spreading of individuals, thus leading to lower competitiveness and narrow distribution.

Hydrotropism as a compensation for high transpiration

Heterogeneous water pattern is common in natural ecosystems. *S. pinnatifolia* individuals had similar transpiration rate as the widespread *S. oblata*, although they had lower root biomass allocation, showing a shift in root biomass from 51% in the homogeneous pattern, as found in Experiment 1 at 35% FC, to 75% in the heterogeneous pattern (approximately 35% FC in water stress pattern), as found in Experiment 2 (Fig. 4 and 7). Thus, the obvious hydrotropism of *S. pinnatifolia* in soils with heterogeneous water distribution suggests that it may be an induced strategy in *S. pinnatifolia* plants to compensate for high foliar water transpiration. In contrast, a symmetrical root distribution with little hydrotropism in *S. oblata*, as shown in Fig. 5 and 6, suggests that the widespread *S. oblata* has higher capacity to conserve water at the leaf level instead of depending on water absorption by roots from the soil.

Hydrotropism is a common strategy for drought adaption in plants grown in arid areas. Hydrotropism, however, is dependent on biomass allocation to belowground tissues at the expense of above-ground components (Fig. 5 and 6), leading to a decrease not only in

photosynthetic leaf areas, but also in lower competitive ability for light and other above-ground resources. Horizontal hydrotropism as observed in *S. pinnatifolia* may be an uneconomical strategy for water uptake from the soil, because horizontal patches of rich water may not be as reliable as those in deep soil, which is always available even under dry conditions. *S. pinnatifolia* seemingly uses two mechanisms, horizontal hydrotropism and deep rooting, to compensate for the limited capacity for water conservation in its leaves instead of homeostasis within the plant to adapt to drought conditions. Shallow rooting in plants has been hypothesized to be positive adaptive responses to low nutrient level in the soil (Gregory, 2006). Similarly, deep rooting and strong hydrotropism could indicate passive adaptive responses to drought and limited homeostasis within the individuals of *S. pinnatifolia*. These results suggest that strong hydrotropism may not be the optimal strategy for adaption to drought in *S. pinnatifolia*. Excessive allocation to belowground components results in reduced allocation to above-ground parts and may comprise its ability to grow sustainably in its native arid habitat. As shown in previous studies, inability to adapt to changing and heterogeneous microhabitats typically lead to narrow distribution and endangered status in plants (Jones, 1985; Kephart and Paladin, 1997).

Greater morphological plasticity means higher energy cost

Plasticity is an adaptation strategy that could potentially help plants better cope with the fluctuating environment in the long term. Energy is required in construction of plasticity, including energy for information detection, acquisition and maintenance. The energy cost, however, will inevitably lead to reduced fitness in the plants (de Witt et al., 1998). As a result,

those plastic behaviors that are more responsive and with lower energetic costs are more beneficial to plants in order to minimize fitness loss (Mooney and Chaiello, 1984).

Physiological plasticity, which has low energy cost, may be more efficient than morphological plasticity to gain a competitive advantage when resource availability is transient (Grams and Anderson, 2007). We found that the narrowly distributed *S. pinnatifolia* had lower physiological plasticity index, particularly in hydraulic conductivity, stomatal conductance and transpiration than the widespread *S. oblata*. *S. pinnatifolia*, in contrast, had higher morphological than physiological plasticity across water regimes from 35% to 95%. These findings suggest that *S. pinnatifolia* preferred a morphological strategy involving biomass allocation between above- and below-ground components to physiological adjustment when soil moisture changed. Under most circumstances, it is relatively more time-consuming and risky to achieve morphological construction, including root hydrotropism, which happened in patched water and required higher energy than physiological adjustments (de Witt et al., 1998). The time-lag between rapid shift in water availability and slow responsiveness of root growth could inevitably cause the temporal mismatch between root construction and the current environment (Givnish, 2002). Results from our experiments suggest that a lower degree of cost-effective physiological plasticity but a higher degree of energy-demanding morphological plasticity in *S. pinnatifolia* likely reduced the fitness of *S. pinnatifolia* plants and restricted the spreading, leading to the narrow distribution of this endangered species.

Summary

Our results demonstrated that higher photosynthetic capacity in the endangered *S. pinnatifolia* is one important trait that enables it to survive in the harsh alpine environment. Higher photosynthetic capacity, however, comes at the expense of greater transpiratory water loss. Low integrative water use efficiency in response to fluctuating water availability reduces the drought adaptability of *S. pinnatifolia* in arid regions. Greater allocation of biomass to roots in rich water regime indicates a mechanistic mismatch in response to abundant resource availability, making it a potentially inferior competitor for above-ground resources such as space and light. Greater horizontal and vertical root hydrotropism in *S. pinnatifolia* in heterogeneous water patches is more likely related to limited interior water conservation as a result of high foliar stomata and root conductance, implying that these mechanisms are compensative water uptake instead of drought-tolerance. Our results demonstrate that *S. pinnatifolia* plants use greater morphological plasticity, which has higher energetic cost than physiological adjustment, to support its water acquisition at all water levels. From our findings, we project that the narrowly distributed *S. pinnatifolia* to be unsustainable to survive in its natural habitats.

Author Contribution Statement

HC, SC and LS conceived of, designed and conducted the study; HC, SC, HH, LS, HZ, ZL, TH and YQ collected data; HC, SC and LS analyzed the data with input from XZ; HC, XZ and LS wrote the paper.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure Legends

FIG. 1. Design of the heterogeneous water treatment in Experiment 2. Each container was divided into three parts of equal volume. The middle part, hatched and marked with a “W”, was watered to field capacity of the soil, whereas the two end parts were not watered during the experiment. One individual of *Syringa oblata* was grown in the left part and one individual of *S. pinnatifolia* was grown in the right part as indicated by a dot in the diagram. The left and right parts were further divided into three vertical sub-sections and marked 1, 2 and 3 respectively. Sub-section 1 was the closest to and sub-section 3 the furthest from the watered middle part. Each sub-section was then partitioned horizontally into three 10-cm layers (a, b and c) at harvest time.

FIG. 2. Hydraulic conductivity (L_{pr}) of the widespread *Syringa oblata* and the endangered *S. pinnatifolia* at each water treatment (% of maximum field capacity). Error bar represents 1 SE ($n=3$). Different letters indicate significant difference at $P < 0.05$ between the two species at each water level.

FIG. 3. Stem biomass (a) and shoot mass fraction (b) of *Syringa oblata* and *S. pinnatifolia* grown at different water levels in Experiment 1. Error bar represents 1 SE ($n=5$). Different letters at the same water level indicate significant difference at $P < 0.05$ among treatments across two species.

FIG. 4. Root mass fraction of *Syringa oblata* and *S. pinnatifolia* grown at different water levels in Experiment 1. Error bar represents 1 SE (n=5). Different letters at the same water level indicate significant difference at $P < 0.05$ among treatments across two species.

FIG. 5. Spatial partitioning of root biomass of *Syringa oblata* and *S. pinnatifolia* at different distances from the central watered area for the heterogeneous water treatment in Experiment 2. The three sections are marked near-water area, middle area and far-from-water area and correspond with the sections marked 1, 2 and, 3, respectively, in Fig. 1. Error bar represents 1 SE (n=3). Different letters indicate significant difference at $P < 0.05$ among treatments across two species.

FIG. 6. Spatial partitioning of root biomass of *Syringa oblata* and *S. pinnatifolia* at different soil layers for the heterogeneous water treatment in Experiment 2. Soil was divided into top, middle and bottom layers, which correspond to sections a, b and c in Fig. 1. Error bar represents 1 SE (n=3). Different letters indicate significant difference at $P < 0.05$ among treatments across two species.

FIG. 7. Comparison of stem mass fraction (SMF) and root mass fraction (RMF) in *Syringa oblata* and *S. pinnatifolia* in Experiment 1 (homogeneous water treatment with 35% of maximum field capacity) and Experiment 2 (heterogeneous water treatment as described in Fig. 1).